REVEALING THE COMPLEXITY OF THE HAIR LIPIDOME

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INTRODUCTION

Hair has the potential as a new, promising matrix in lipidomics studies. The **long detection window** of weeks to months provides the opportunity to monitor metabolomic alterations over a longer timeframe with the potential to identify small molecules that play a key role (early) in chronic conditions.

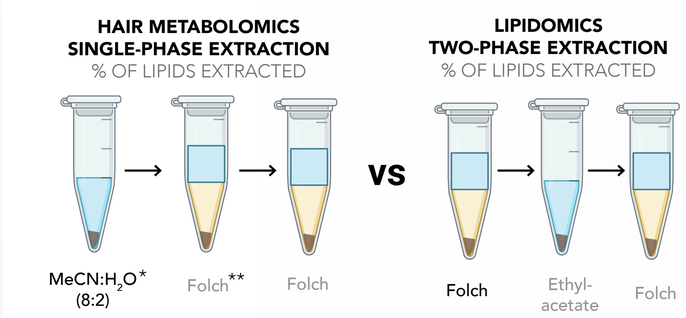
Current untargeted hair metabolomic studies rely on forensic hair analysis methodologies detecting polar (exogenous) compounds. Nevertheless, lipids play an essential role in various chronic diseases. However, up to date, there is no comprehensive and accurate identification of the hair lipidome.

OBJECTIVES

1. PROVE THE POTENTIAL OF HUMAN HAIR AS NOVEL MATRIX IN LIPIDOMICS...
2. INVESTIGATE THE IMPACT OF SAMPLE PREPARATION FACTORS ON LIPID ABUNDANCE...
3. ESTABLISH THE GLOBAL COMPOSITION OF THE HAIR LIPIDOME...

METHODS

1 ...BY COMPARING A SINGLE-PHASE EXTRACTION (USED IN HAIR METABOLOMICS) WITH A LIPIDOMICS EXTRACTION TECHNIQUE



* Eisenbeiss et al. 2019. ** Folch: $CHCl_3$:MeOH:H_2O, 8:4:3, v/v/v.

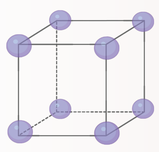
2 ... BY PERFORMING A FRACTIONAL FACTORIAL DESIGN EXPERIMENT

Factor	Level 1	Level 2
Pulverization time	3 min	10 min
Extraction solvent*	Matyash	Folch
Addition of AOX**	Yes	No
Sample-to-solvent ratio	1:30	1:120
Incubation technique	Shaking @ 20°C	On ice
Extraction time	30 min	240 min

*Matyash: MBTE:MeOH:H_2O, 10:3:2.5, v/v/v.

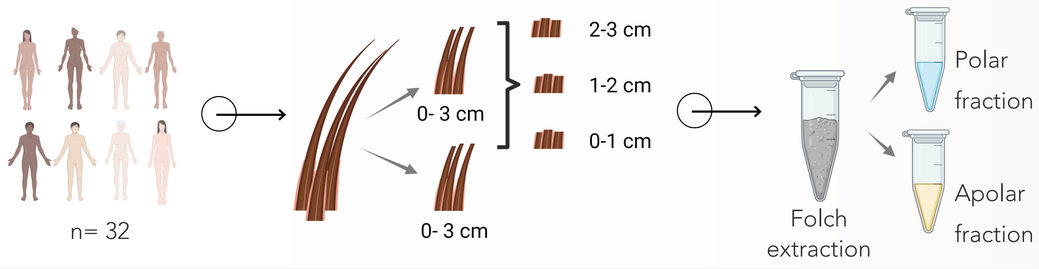
** 1 mM $(NH_4)_2$ EDTA, 0.5 mM ascorbic acid and 1 mM BHT

16 experiments in duplicate



3 ... BY DETERMING THE STABLE HAIR LIPIDOME USING AN UNTARGETED APPROACH

STUDY DESIGN



DATA ACQUISITION

LC
Column: ACQUITY UPLC BEH C18 (150 x2.1 mm, 1.7 µm)

MP A*: 5 mM NH_4COOCH_3 in H_2O/MeCN (7/3, v/v)
MP B*: 5 mM NH_4COOCH_3 in H_2O/MeCN/IPA (2/10/88, v/v/v)

IM-QToF-MS
ESI +/-
DDA, iterative MS
DTIMS

*ESI +: MP A and B + 0,1% (v/v) $HCOOCH_3$

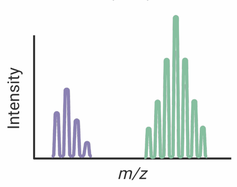
DATA ANALYSIS

[1.] Software acquired annotations

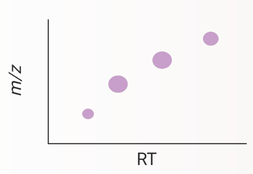
MS-DIAL
LIPOSTAR*
LIPIDMATCH*
LIPIDHUNTER*

*Future perspective

[2.] Manual curation*
Fragmentation
Isotopic pattern

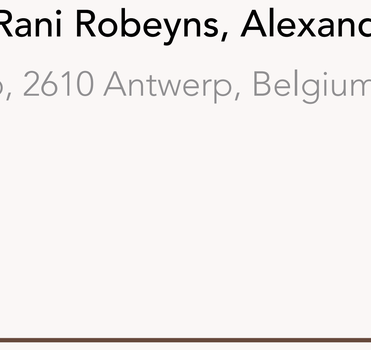


[3.] RT mapping*



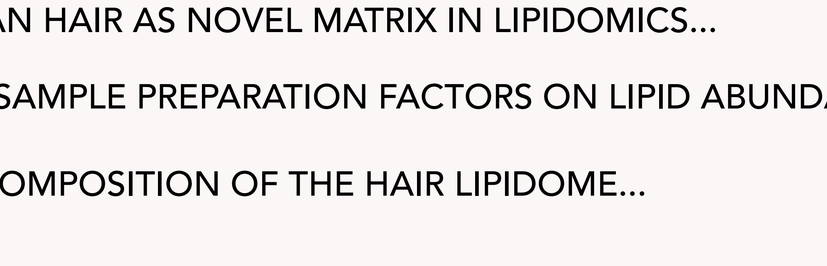
RESULTS

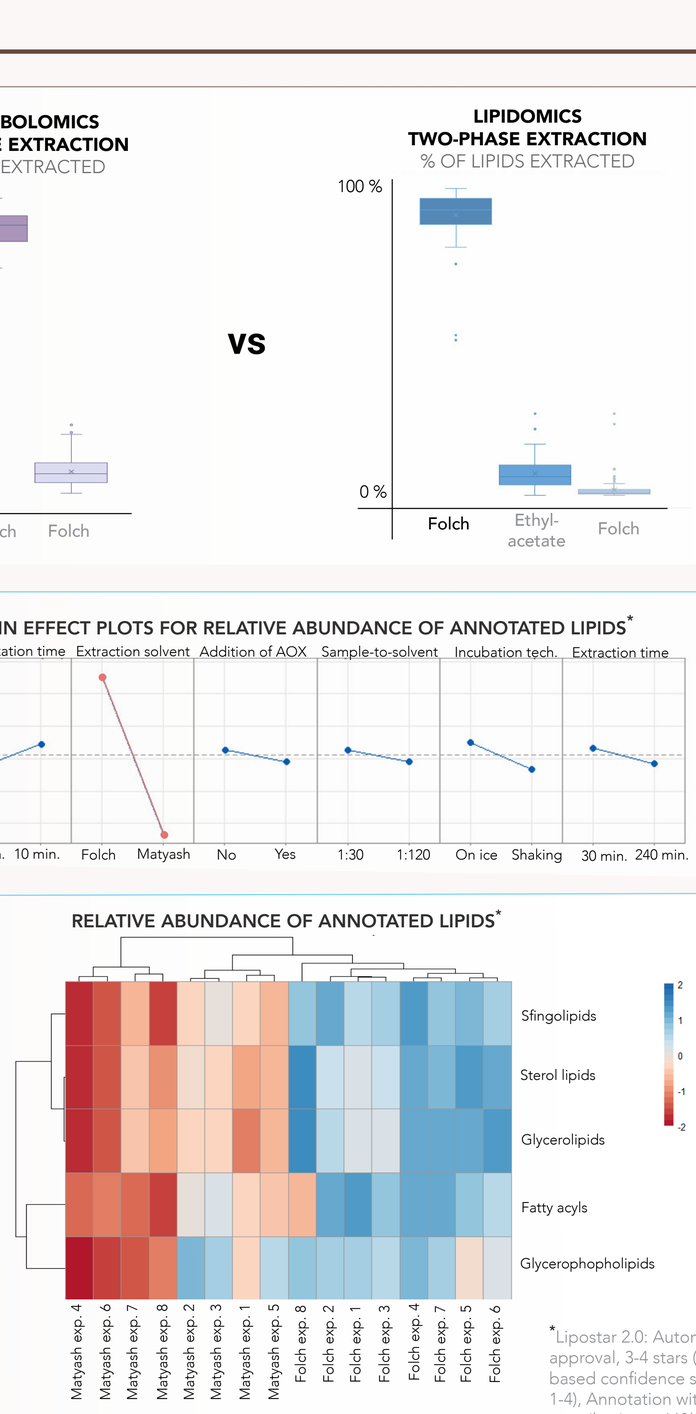
1



vs

2

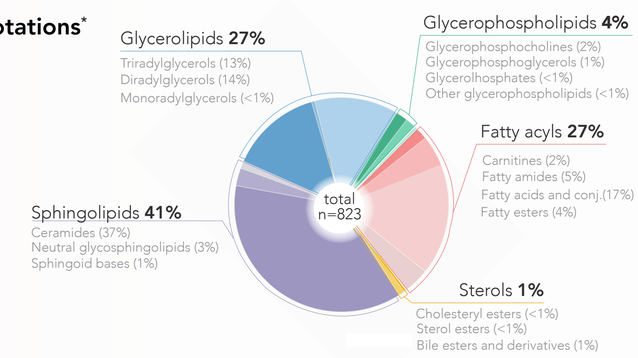




*Lipostar 2.0: Automatic approval, 3-4 stars (Lipid rule-based confidence system 1-4), Annotation with a blank contribution < 11%, RSD QC <30%

3

MS-DIAL annotations*



* Based on LC-MS/MS data. Annotations with a blank contribution < 11%, RSD QC <30%

CONCLUSION

A significant increase in the percentage of extractable lipids is uncovered using a lipidomics-based extraction method.

The type of extraction solvent has a significant impact on lipid signal intensities: The Folch extraction procedure is the preferred extraction method to detect low-abundance lipids in hair.

Sphingolipids make up 41% of the hair lipidome followed by fatty acyls (27%), glycerolipids (27%), glycerophospholipids (4%) and sterols (1%).

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